Standard Operating Procedure for Benthic Invertebrate Laboratory Analysis

LG407

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1.0 SCOPE AND APPLICATION

1.1 The procedures outlined here are for the sorting, identification, and storage of benthic invertebrates in samples collected from the Great Lakes National Program Office surveys.

2.0 SUMMARY OF METHOD

2.1 This procedure explains how to 1) prepare samples for processing, 2) separate animals from sediment, 3) prepare, record, and maintain specimens for taxonomic analysis and storage, and 4) perform required QA/QC steps.

3.0 EQUIPMENT LIST

3.1 Equipment

Scintillation vials (5-mL)
Larger storage Jars (for larger or numerous specimens)
Preserved field samples (1-L)
Petri dishes, gridded
Forceps (fine points, 2 pair per worker)
Spoon
Wash bottle
Temporary storage jars
Dissecting microscope
Compound microscope
Slides (25 x 75 mm)
Coverslips (22 x 22 mm)
Slide storage cases
Taxonomic keys (section 12.0)

3.2 Reagents

70% Ethanol (EtOH)
Mounting media (CMC-9, CMC-10, or Hoyer's)
Formalin (3.7% formaldehyde)

4.0 SORTING PROCEDURES

4.1 Label a group of scintillation vials for each replicate field sample. Labels for the laboratory sample vials must include the sample number, sample date, lake and station location number (e.g., MI 47), and station replicate (A, B or C). There should be a scintillation vial for each major taxonomic group present (amphipods, oligochaetes, chironomids, etc.) for each replicate field sample.

4.2 Sorting benthic invertebrates

4.2.1 Rinse the formalin out of the sediments by placing the entire sample into a 500-mm sieve within a tray/pan. Dispose of formalin using proper procedure. Under the hood, run tap water over the sample until the formalin is gone. If there were multiple bottles for a single sample (check field sample log) they can be combined at this point, or they can be processed separately; be sure to label all jars properly (e.g., 1 of 3, 2 of 3, 3 of 3). However, keep the replicate samples for each station separate.

4.2.2 Rinse the entire sample from the sieve and into a 16-oz glass jar or other temporary receptacle. Label the jar exactly as the original jar is labeled.

4.2.3 Under a dissecting microscope, remove organisms from the sediment, and transfer them to the appropriately labeled vial (see 4.1). It will be necessary to do this by spooning a small amount of sediment into the gridded Petri dish and then using the dissecting microscope to locate all appropriate organisms.

4.2.4 Place spent sediments back into the sample bottle with 10% formalin solution for further QC picking. Be sure the label on the bottle is correct.

5.0 MOUNTING PROCEDURES

5.1 General mounting guidelines

5.1.1 Place invertebrates on the slides length-wise. An attempt should be made to mount specimens of the same size on a slide. Mounting thick bodies with thinner bodies will make the latter hard to see. When possible, avoid allowing specimens to cross each other or wrap over on themselves. This may make the mount too thick and cause excessive air bubbles in addition to obscuring anatomy/morphology necessary for identification

5.1.2 Labeling is done directly on the slide using a "superfrost" permanent marker for both oligochaetes and midges.

5.1.3 CMC-9 (low viscosity) and CMC-10 (high viscosity) mounting media can be mixed to provide the best thickness for the animals being mounted. In addition, Hoyer's mounting medium may be used.

5.2 Mounting Oligochaetes

5.2.1 Most oligochaetes will be mounted according to Appendix A in the Guide to the Freshwater Oligochaetes of North America (Kathman and Brinkhurst, 1998). Exceptions may include the following:

Lumbriculidae: *Stylodrilus heringianus* Claparède, 1862, (a common species, usually the only species at great depths, in the Great Lakes)
Naididae: *Stylaria lacustris* (Linnaeus, 1767) (a distinctive species with a proboscis); *Dero* sp. (distinctive group with posterior gills)

Representative specimens of the above 3 taxa should be slide mounted for each site, but since individuals of these taxa can be identified using a dissecting microscope, the remaining specimens can be stored in a labeled vial.

5.2.2 Mount 5 animals per coverslip, 2 cover slips per slide.

5.2.3 Label the slide with sample number, station, replicate number, sample date, taxonomic group (Oligochaete or Chironomid), and number in the slide sequence for that replicate.

e.g.: 99GB49S36
HU 32a
16 Aug 99
Oligochaetes
1of 6

5.2.4 Fragments of oligochaetes should be counted and placed in a separate vial. Fragments are specimens that do not have the anterior portion of the body (head). If it is not clear whether a specimen possesses its head, assume that it does possess its head, and mount the specimen for identification.

5.3 Mounting Chironomids

5.3.1 Larval chironomids should be mounted according to the publication Identification Manual for the Larval Chironomidae (Diptera) of North and South Carolina (Epler, 2001). However, easily identifiable taxa may be identified using a dissecting microscope, with individuals subsequently stored in labeled vials. Taxa include: *Chironomus, Cryptochironomus, Procladius.* Additional taxa can be included in this list, but representative specimens of the above 3 taxa and any additional taxa should be slide mounted.

5.3.2 Mount 3 animals per coverslip, 2 cover slips per slide. Mount the body as well as the head capsule. If the body is too large, it may be preserved in a vial; the vial and slide should be cross referenced.

5.3.3 Labeling should be the same as oligochaete slides (see 5.2.3).

6.0 TAXONOMIC ANALYSIS PROCEDURES

6.1 These procedures should be used for amphipods and all other non-slide mounted animals.

6.1.1 Identify the specimens and record the number of specimens in each taxon on the appropriate bench sheet. Only specimens that possess their heads should be included for identification and enumeration, even if only the head is the piece that is present; all other parts, pieces, exuviae, and empty shells should be ignored.

EXCEPTION: If enough of the body of a specimen (excluding exuviae and empty shells) is present to make a valid identification, and it can be absolutely assured that other body parts of the specimen are not present in the sample (i.e. the specimen is in pieces and is being counted more than

once), then the piece may be included in the taxonomic analysis and enumeration. However, this is not a common situation.

6.1.2 Once this is completed, return all the animals to their scintillation vial, seal and label for archived sample storage.

7.0 SAMPLE PRESERVATION, LABELING, AND STORAGE

7.1 Slide-Mounted Organisms: Mounted chironomids and oligochaetes will be stored as archived samples.

7.2 Sorted invertebrates: Once the amphipods and other non-oligochaete/chironomid animals are counted and identified, all animals can be archived in their properly labeled scintillation vials with 70% ethanol.

7.3 Sediment: The remainder of the sample, which may contain sediment, zebra mussels, nematodes, and other non-target organisms should remain preserved in the original sample bottle with formalin until proper QC checks are completed. After completion of QC checks, the sample may be properly discarded.

7.4 After analysis is complete and data has been submitted and approved by the WAM, all archived samples should be stored at the GLNPO storage facility at 536 S. Clark Street, Chicago IL.

8.0 DATA HANDLING AND CALCULATIONS

8.1 The data must be converted from estimated raw sample counts to abundance per m^2 for each species by multiplying the totals by the number: 19.12. This conversion factor is derived from dimensions of the Ponar grab sampler. If a different sampler is used the factor should be recalculated.

8.2 Calculate a mean abundance for each species and report the mean of each species (or lowest taxonomic level) by Station Number.

9.0 SAFETY AND WASTE HANDLING

9.1 Personal protection equipment (safety glasses, gloves and lab coat) should be worn in the laboratory while preparing and handling samples for analyses.

9.2 All samples preserved with formalin should be handled under the hood prior to being rinsed for sorting (Section 4.2.1).

9.3 Follow laboratory waste disposal guidelines regarding formalin solution waste. Sample waste should be emptied into a waste container.

10.0 TRAINING AND QUALITY CONTROL

10.1 New analysts are required to receive formal training in the areas of terminology, anatomy, morphology, and taxonomy of Great Lakes benthic invertebrates. This can be accomplished in one of two ways: instruction from a senior benthic analyst in the laboratory, or by attending an external course taught by benthic specialists.

10.1.1 Acceptable training courses can be found by contacting either the North American Benthological Society or the International Association of Great Lakes Research.

10.2 Quality Control Checks on the processing of benthic macroinvertebrates

10.2.1 Picking samples:

The senior taxonomist or the lead assistant will "second pick" at least 10% of the samples picked by each assistant. One sample from a block of 10 consecutively picked samples by each person will be randomly chosen for second picking. Error percentages in picking should be less than 10%, preferably less than 5%.

However, samples that yield very low numbers of individuals can lead to high percentages of error (e.g., missing 1 specimen from a total of 4 specimens equals a 25% "picking error"). High error percentages from these types of samples will be taken into consideration when determining if the sample passes or fails the QC check. The main criteria in this determination will be deciding whether the error affects the ecological interpretation of the data.

Samples that do not pass the QC check will lead to the repicking of another sample within the assistant's block of 10 samples. If the second sample fails the QC check, all of the samples within the block of 10 will be repicked by the same assistant. Another round of QC checks will be repeated on the block of 10 samples.

During the initial training period of an assistant, additional samples will be 2nd picked until it is assured that the assistant is effectively removing 90-100% of the total number of organisms.

10.2.2 Taxonomy:

Most, if not all, of the taxonomic identifications will be done by the senior taxonomist. Identifications of only amphipods may also be accomplished by the lead assistant.

Unusual or difficult oligochaetes may be sent to the lab at Aquatic Resources Center, College Grove, Tennessee, for expert identification.

10.2.3 Enumeration:

All counts of specimens will be made by the senior taxonomist (or lead assistant) when the specimens are identified. The lead assistant will "second count" 10% of the samples from the senior taxonomist, and vice versa. There must be a 98% agreement on counts. Samples that fail the QC Check will result in all of the samples from the block of 10 samples to be recounted.

11.0 TAXONOMIC REFERENCES

11.1 Primary Taxonomic Sources for Identification and Nomenclature

Merritt, R.W. and K.W. Cummins (eds.). 1996. An Introduction to the Aquatic Insects of North American. 3rd Edition. Kendall/Hunt Publishing Co., Dubuque IA.

Pennak, R.W. 1989. Fresh-water Invertebrates of the United States. Porifera to Mollusca. 3rd Edition. John Wiley & Sons, Inc. New York, NY.

Smith, D.G. 2001. Pennak's Freshwater Invertebrates of the United States. Porifera to Crustacea. 4th Edition. John Wiley & Sons, Inc. New York, NY.

Thorp, J.H. and A.P. Covich. 2001. Ecology and Classification of North American Freshwater Invertebrates. 2nd Edition. Academic Press. San Diego, CA.

11.2 Additional Taxonomic Sources for Species Identification

11.2.1 Insecta: Diptera: Chironomidae

Epler, J.H. 1995. Identification Manual for the Larval Chironomidae (Diptera) of Florida. Final Report for DEP Contract Number WM579. Florida Department of Environmental Protection, Tallahassee.

Epler, J.H. 2001. Identification Manual for the Larval Chironomidae (Diptera) of North and South Carolina. North Carolina Department of Environment and Natural Resources.

Oliver, D.R, M.E. Dillon. 1994. Corrections and additions to "A Catalog of Nearctic Chironomidae." Proceedings of the Entomological Society of Washington 96: 8-10.

Oliver, D.R, M.E. Dillon , and P.S. Cranston. 1990. A Catalog of Neararctic Chironomidae. Research Branch, Agriculture Canada Publication No. 1857/B: 89pp.

Wiederholm, T. (ed.). 1983. Chironomidae of the Holarctic region -- keys and diagnoses. Part 1. Larvae. Entomologica Scandinavica Supplement No.19. pp 457.

11.2.2 Insecta: Ephemeroptera

McCafferty, W.P. 1975. The burrowing mayflies of the United States (Ephemeroptera: Ephemeroidea). Transactions of the American Entomological Society 101:447-504.

11.2.3 Insecta: Trichoptera

Wiggins, G.B. 1996. Larvae of the North American Caddisfly Genera (Trichoptera). 2nd Edition. University of Toronto Press, Inc. Toronto, Ontario.

11.2.4 Annelida

Kathman, R.D. and Brinkhurst, R.O. 1998 (Revised 1999). Guide to the Freshwater Oligochaetes of North America. Aquatic Resources Center, College Grove, Tennessee.

Klemm, D.J. 1985. A Guide to the Freshwater Annelida (Polychaeta, Naidid and Tubificid Oligochaeta, and Hirudinea) of North America. Kendall/Hunt Publishing Co. Dubuque, Iowa.

11.2.5 Mollusca

Burch, J.B. 1982. Freshwater Snails (Mollusca: Gastropoda) of North America. EPA-600/3-82-026. U.S. EPA, Cincinnati, Ohio.

Mackie, G.L., D.S. White, and T.W. Zdeba. 1980. A Guide to the Freshwater Mollusks of the Laurentian Great Lakes with Special Emphasis on the Genus, *Pisidium*. EPA-600/3-80-068. U.S. EPA, Duluth, MN.

11.2.6 Amphipoda

Bousfield, E.L. 1958. Fresh-water amphipod crustaceans of glaciated North America. The Canadian Field-Naturalist 72: 55-113

Holsinger, J.R. 1972. The Freshwater Amphipod Crustaceans (Gammaridae) of North America. U.S. EPA Biota of Freshwater Ecosystems, Identification Manual No. 5.

Witt, D.S., P.D.N. Hebert, and W.B. Morton. 1997. *Echinogammarus ischnus*: another crustacean invader in the Laurentian Great Lakes basin. Canadian Journal of Fisheries and Aquatic Sciences 54: 264-268.